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Biocompatibility testing of coated titanium and ceramic implants in a pelvic model in sheep

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Dedicated to my family

INDEX

Zusammenfassung:

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Biokompatibilität von beschichteten Titanium und Keramik Implantaten in einem Beckenmodell beim Schaf

Ziel: Ziel dieser Studie war die histologische und biomechanische Evaluierung von vier neuen experimentellen Oberflächenbeschichtungen im Vergleich zu zwei unbeschichteten Referenzimplantaten. **Material und Methoden**: Insgesamt wurden 72 Implantate in die Hüftknochen von vier Schafen eingesetzt. Es wurden drei unterschiedliche Beschichtungen (A=Tantal. Ta; B=Titanium1. Ti1; C=Titanium1+ Hydroxyapatit. Ti1+HA) eines Titan-Implantates (Ti6Al4V) sowie eine Beschichtung (D=Titanium2. Ti2) eines Keramik-Implantates (Zirkonium-verstärktes Aluminium; ZTA) getestet. Als Referenzen wurden zwei unbeschichtete Implantattypen (Titan und Keramik) verwendet. Zwei Floreszenz-Markern zu zwei unterschiedlichen Zeitpunkten wurden appliziert. Die Probengewinnung fand nach acht Wochen statt. Implantate wurden histologisch (BIC) und biomechanisch (push out Test) analysiert. **Resultate**: Alle beschichteten Implantate zeigten im Vergleich zu den beiden Referenzen hohe Push-out (>629.76 Ncm) und BIC (> 27.21%) Werte. Die Titan-Implantate mit der Beschichtung C (Ti1+HA) zeigten die höchsten biomechanischen und histologischen Werte vergleichend zu den Referenzimplantaten (BIC $p<0.001$, Push out Test $p<0.008$). **Schlussfolgerung**: Diese Studie hat gezeigt, dass eine raue und poröse Beschichtung von Implantaten eine schnellere und stärkere Osseointegration im Vergleich zu unbeschichteten Implantaten bewirken kann.

Stichworte:
Biokompatibilität, beschichtete Implantate, Osseointegration, Titanium, Keramik

Summary:

Vetsuisse-Faculty University of Zurich 2019
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Biocompatibility testing of coated titanium and ceramic implants in a pelvic model in sheep

Purpose: The aim of this study was to histologically and biomechanically assess the osseointegrative capabilities of four novel implant coatings using a pelvic implantation model in sheep. **Methods:** A total of 72 implants were tested in four sheep. Three different surface coatings: A=Tantalum (Ta) , B=Titanium (Ti), C=Titanium + Hydroxyapatite (Ti+Ha) for titanium-based implants and one type of surface coating (D=Ti) for ceramic-based implants were tested. Two types of uncoated reference items (Ti=RI1 or ceramic=RI2) were used as reference. Bone remodeling over time was illustrated using two different fluorescence dyes. Samples were evaluated histologically and biomechanically. **Results:** All test items (TI) yielded high results in the bone to implant contact (BIC) evaluation (>27.21%) and in the push out tests (>629.76 Ncm) compared to the RI's. TI-C (Ti + HA) yielded the highest histological (49,90±13,36%) and biomechanical (1038,85±385,16) means compared to the RI's with a high statistical significance (BIC $p<0.001$, Push out test $p<0.008$) as well as demonstrating a tendency of higher values among all TI's. **Conclusions:** All four coating materials used in this study showed enhanced osseointegration. Furthermore, titanium implants coated with a combination of titanium and hydroxyapatite (TI-C) showed a tendency of higher values for osseointegration.

Keywords: Biocompatibility, implant coatings, osseointegration, titanium & ceramics, sheep pelvic model

Biocompatibility testing of coated titanium and ceramic implants in a pelvic model in sheep

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ABSTRACT

Purpose: The aim of this study was to histologically and biomechanically assess the osseointegrative capabilities of four novel implant coatings using a pelvic implantation model in sheep.

Methods: A total of 72 implants were tested in four sheep. Three different surface coatings: A=Tantalum (Ta) , B=Titanium (Ti), C=Titanium + Hydroxyapatite (Ti+Ha) for titanium-based implants and one type of surface coating (D=Ti) for ceramic-based implants were tested. Two types of uncoated reference items (Ti=RI1 or ceramic=RI2) were used as reference. Bone remodeling over time was illustrated using two different fluorescence dyes. Samples were evaluated histologically and biomechanically.

Results: All test items (TI) yielded high results in the bone to implant contact (BIC) evaluation (>27.21%) and in the push out tests (>629.76 Ncm) compared to the RI's. TI-C (Ti + HA) yielded the highest histological (49,90±13,36%) and biomechanical (1038,85±385,16) means compared to the RI's with a high statistical significance (BIC $p<0.001$, Push out test $p<0.008$) as well as demonstrating a tendency of higher values among all TI's.

Conclusions: All four coating materials used in this study showed enhanced osseointegration. Furthermore, titanium implants coated with a combination of titanium and hydroxyapatite (TI-C) showed a tendency of higher values for osseointegration.

Keywords: Biocompatibility, implant coatings, osseointegration, titanium & ceramics, sheep pelvic model

Running Heads: Biocompatibility testing of coated titanium and ceramic implants.

INTRODUCTION

Increases in life expectancy and in quality of health care lead to a continuous demand for innovative developments and basic research in implant materials and technologies. New implants have to demonstrate advanced characteristics towards existing ones. It has become clear that indications and limitations play an important role when choosing an implant material over another for a specific application, as they do not follow the one-size-fits-all principle[1]. It is only naturally then, that numerous types and variations of implant materials were tested and also used during the years.

Due to their convincing results, metal implants, especially titanium and its alloys have achieved an approved standard in the field of dental and orthopedic surgery[1]. Titanium implants are well known for their good biocompatibility[2], long-term stability[3,4], low rates of erosion and corrosion[5] as well as excellent osseointegration[2,6].

Yet titanium implants in dental implantology display some disadvantages too. Due to its relatively low stiffness, commercial pure titanium (cp-Ti) shows a relatively low resistance to tribocorrosion[7]. Especially the greyish color of the metal often can result in compromised aesthetics in case of a thin gingiva or a dehiscence in the frontal region[8]. Additionally, titanium implants distort or even falsify radiological evaluations of the periimplant region[9,10]. Recent reports also suggest that titanium alloys respectively titanium particles may lead to allergic reactions in rare occasions[11].

By contrast, ceramic implants offer beneficial properties in this respect. They have been successfully used for decades, often for joint reconstructions[12]. It is only in recent years though, that ceramics are gaining an increasing popularity in implant manufacturing, especially zirconia ones[13]. The later in fact, demonstrate excellent results in terms of biocompatibility[2,14], good wear resistance[15], and osseointegration[2,6,14]. Ceramic dental implants also present an aesthetic advantage due to their natural, tooth-like color[8]. This material also does not interfere with x-rays. Nevertheless, more data on long-term stability is still required [16].

As mentioned above both titanium and ceramic have proven to achieve good osseointegration.

This term was originally proposed by Brånemark et al.[17] and refers to the direct interface between the implant surface and its surrounding tissue. Ideally this should be newly formed bone tissue, serving as a stabilizing anchor against early mechanical loads, implant loosening and failure[18].

As recognized by Albrektsson T. et al. already 30 years ago, the success of osseointegration depends on numerous parameters. The most important ones are implant location, host bone quality, mechanical load, surgical techniques and implant design[19-22]. The latter is largely influenced by its surface character, which is determined mainly by its topography[20,23], bioactive potential[24] and physical properties[25]. A key factor in this respect is a porous or rough surface modification[26]. An increased 3D interface area simply offers more contact area for bone tissue to grow into and therefore can enhance the shear strength resistance[27]. Thus, surface modification of titanium and ceramic implants has proven to increase their performance as well as their long-term success[20,28,29].

Besides physical or chemical surface modification also special coating procedures with highly biocompatible materials have been tested and used to improve the success rate of implants even more[30]. Hydroxyapatite (HA) is frequently used to enhance the osseointegrative potential of an implant[31,32] and has been a subject of numerous research projects. One key characteristic of HA is its bone-like composition, giving it stronger osseointegrative properties than many other implant materials[33,34].

Nevertheless, popular materials such as Ti and Ceramics nowadays share their spotlight with another promising alternative- tantalum. So far Tantalum has proven to have desired characteristics such as excellent biocompatibility[35], excellent corrosion–erosion resistance[36,37], high shear strength[38] and high porousity[39]. Furthermore, porous tantalum has delivered promising results in promoting osseointegration and bone ingrowth[24,38]. However, high porosity also potentially means increased inner-implant fluid flow, which in turns might enhance the risk of wearing, debris detachment and consequently aseptic loosening. Regarding this aspect, as well as regarding the reactivity of such Ta-particles, little is known[40]. The aim of this study, therefore, was to assess the osseointegrative capabilities of four novel coating layers: three different surface coatings for titanium-based implants (machined from Ti6Al4V) as well as one type of surface coating for ceramic-based implants (zirconia toughened alumina, ZTA). They were compared with uncoated implants as references.

MATERIAL AND METHODS

Study Design

The aim of this study was to examine the osseointegrative properties of four different newly developed surface coatings, compared with two uncoated implants as references (RI). The implant bulks consisted of either titanium or ceramic.

A standardized and previously well-described pelvic implantation model in sheep was chosen[41]. Four adult, female, Swiss Alpine sheep were used, all with an age of three years (74 months) and an average weight of 71.4 kg (68-78.2 kg).

Eighteen implants per sheep were bilaterally implanted in the pelvis, nine on each side. They were placed in alternating order along the linea glutea posterior (Fig. 1) Custom made fluorescent dyes were applied at different time points to dynamically represent new bone formation and remodeling.

Eight weeks after surgery the animals were sacrificed and all implants were harvested. Altogether, 36 implants (six of each type) were processed for histological analysis, using native sections for fluorescence evaluation and surface stained ones for histomorphometry.

An equal number of implants was biomechanically tested using a push out method (N=72).

This study was conducted according to the Swiss regulations of Animal Welfare. Permission was granted by the local federal authorities (#ZH004/16).

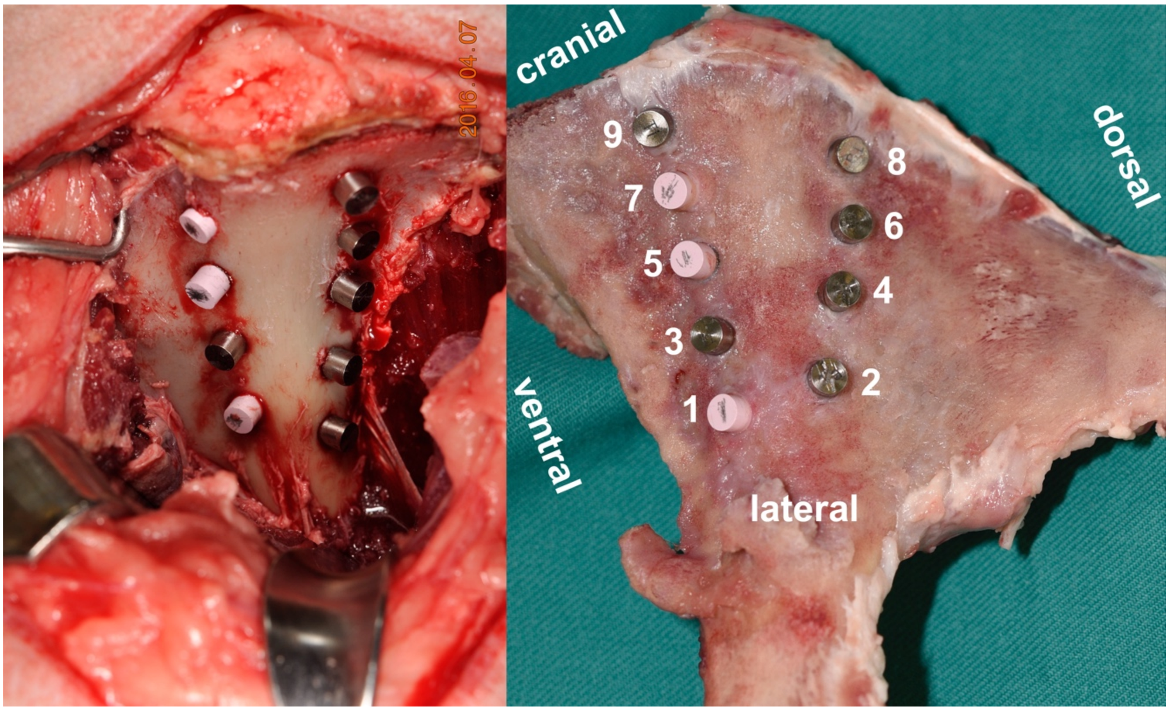

Fig.1: This figure represents the implantation scheme used for this pelvic implantation model in sheep. The right picture demonstrates the positions along the iliac, marked with the numbers 1-9. The left figure shows the surgery field of the right lateral pelvis bone immediately after implantation of the nine implants.

Biomaterials

Six types of implants were provided by Eurocoating Spa (Pergine Valsugana, Trento, Italy).

Four were made out of a titanium 6-aluminium 4-vanadium alloy (Ti6Al4V) and two were produced out of zirconia toughened Alumina (ZTA) ceramic (Fig. 2).

Four new surface coatings were developed for the purpose of this study. The tested implants and their features are presented in the table below (Tab. 1).

Implants were custom made and manufactured in a cylindrical form, 15 mm in length and with a diameter of 5 mm (a total of 5.1-5.2 mm with the coating).

For the coating process, normal controlled atmosphere plasma spraying technology (coating A+B), air plasma spraying technology (coating C), or vacuum plasma spraying technology (coating D) were used. Depending on the coating process itself and different starting powder sizes, the implants consisted of three different characteristics for surface roughness and porosity as shown in table 2. TI-A, -B and -C had high roughness and medium-high porosity, whereas TI-D had low roughness and porosity. The uncoated RI-1 and -2 had very low roughness and porosity.

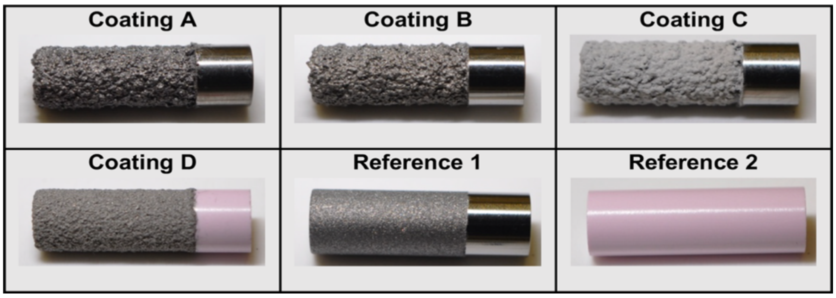

Fig.2: This figure shows the TI's A-D (A having a Ti6Al4V bulk and baring a tantalum coating; B consisting of a Ti6Al4V bulk with titanium coating type 1; C consisting of Ti6Al4V bulk with a Titanium coating type 1 plus a second layer of hydroxyapatite on top of it; D consisting of a ZTA-ceramic bulk and with a titanium coating type 2), as well as the uncoated RI-1 (made out of Ti6Al4V) and -2 (made out of ZTA-ceramic). Pictures were taken prior to implantation.

Group	Number of coating layers	Substrate Material	Coating Material	Coating technique	Average thickness [μm]	Porosity [%]	Roughness [Ra, μm]
TI-A	1	Ti6Al4V	Ta	CAPS	500	47.7±1.7	51.3±2.7
TI-B	1	Ti6Al4V	Ti,cp1	CAPS	500	40.3±2.5	67.9±7.7
TI-C	2	Ti6Al4V	Bond layer: Ti,cp1 Top layer: HA	Bond layer: CAPS Top layer: APS	Bond layer: 500 Top layer: 80	Bond layer: 40.3±2.5 Top layer: 5.3±1.5	52.87±5.37
TI-D	1	ZTA	Ti,cp2	VPS	350	39.5±4.2	18.1±1.1
RI-1		Ti6Al4V	-	-	-	-	-
RI-2		ZTA	-	-	-	-	-

Tab.1: This table describes the different implant types and their features including composition, coating technique and surface characters.

Anesthesia

One week prior to surgery the sheep were brought to the research facilities for acclimatization. For premedication xylazine (0.1 mg/kg BW i.m., Xylazin Streuli, Streuli Pharma AG, Uznach, Schweiz) and buprenorphine (0.01 mg/kg BW i.m., Temgesic® Reckitt Benckiser AG, Walliselen, Switzerland) were used. The later was administrated four times in total every four hours for analgesia. In addition, the animals received carprofen (4 mg/kg BW i.v., sid for 5 days, Rimadyl®, Pfizer, Vertrieb Dr. Graeub AG. Zurich. Switzerland), antibiotics (procain-penicillin, 30.000 I.U i.v., bid for five days, Streuli ad us. vet., G. Streuli & CO. AG, Uznach, Switzerland; and gentamicine 4 mg/kg BW i.v., sid for five days, Vetagent® ad us. vet., Veterinaria AG, Zurich, Switzerland) as well as a single injection of tetanus-serum (3000 I.E. s.c., Tetanus-Serum Intervet, MSD Animal Health, Luzern).

For anesthesia, a standardized protocol was used. Induction was achieved with ketamine (3-5 mg/kg BW i.v., Narketan® 10, Vetoquinol AG, Belp-Bern, Switzerland), diazepam (0.1 mg/kg BW i.v., Valium®, Roche Pharma AG Kabi AG, Reinach, Switzerland) and propofol (0.4-0.6 mg/kg BW i.v., Propofol® 1% Fresenius, Fresenius Kabi AG, Stans, Switzerland), the later administered to effect.

After intubation, anesthesia was maintained with a balanced anesthetic protocol using inhalation anesthesia (Isoflurane 1-1.5 Vol%, Attane Isoflurane ad us. vet., Minard INC. Orchard Park, NY, USA), intravenous constant rate infusions of propofol (0.1-0.8 mg/kg BW/min), ketamine (20-40 μg/kg BW/min) as well as fluid application (lactated Ringer's solution 5ml/kg BW/h, Braun Medical AG, Sempach, Switzerland).

For optimal analgesia, an epidural anesthesia at the foramen lumbosacrale was performed using morphine (0.1 mg/kg BW diluted with sterile physiologic saline solution up to a total of 2 ml, Morphin-HCL, Amino AG, Neuenhof, Switzerland).

During surgery, monitoring of the animals was achieved using ECG, capnography, pulse oxymetry and invasive arterial measurement of the blood pressure.

Surgery postoperative care

The surgical procedure was performed according to a previously well-described method[41]. Pictures of the operation field can be found under Fig. 1.

After surgery, sheep were housed in small pens to reduce the risk of implant failure and were monitored and fed twice daily until their sacrifice 8 weeks post-surgery.

Intravital fluorescence markers

In order to follow bone remodeling over time, fluorescence dyes were injected subcutaneously. First calcein green (5 mg/kg BW, Fluka AG, Buchs, Switzerland) was administered four weeks post surgery and xylenol orange (90 mg/kg BW, Fluka AG, Buchs, Switzerland) three days prior to sacrifice.

Sample harvesting

Immediately after sacrifice, the pelvis was harvested. Radiographs of the pelvis were taken for documentation purposes using a Faxitron (HP Cabinet X-Ray System, Hewlett&Packard, Kodak France). The surrounding tissue was inspected for signs of inflammation or hematoma. Implants were cleansed of any covering tissue, identified and manually assessed for stability or loosening. Subsequently, bone blocks (app. 1x1 cm) containing both, implants and adjacent bone tissue (figure 3), were harvested using a band saw (Stryker® Instruments. Michigan. USA). Specimens intended for histology were immediately placed in 40% ethanol, whereas those intended for the biomechanical testing were enwrapped in moist gauzes, sealed in plastic bags and kept cool (4°C) until testing a couple of hours later.

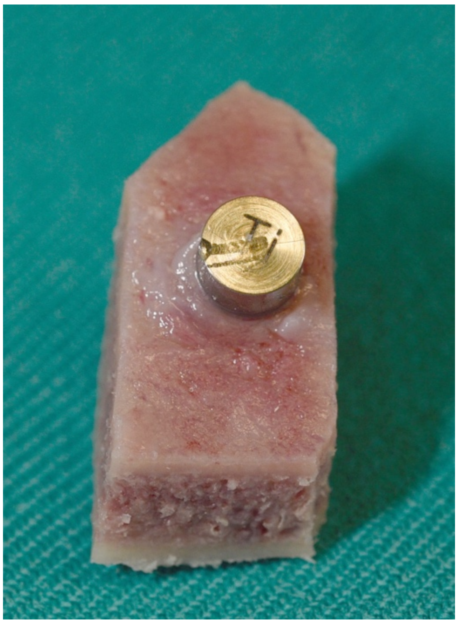

Fig.3: This figure shows a bone sample with an implant after sacrifice and preparation of the single bone-implant blocks for further testing.

Histology

Histological preparations

Histological preparation occurred according to a standardized, previously described method[42]. Briefly, histological sections made out of polymerized MMA blocks were cut to either serve as native slides for fluorescence (mounted on transparent slides) or additionally surface-stained with toluidine blue to serve as stained sections (mounted on acropal slides). Before staining microradiographs of the ground sections were taken for documentation purposes (Figure 4-1, 4-2).

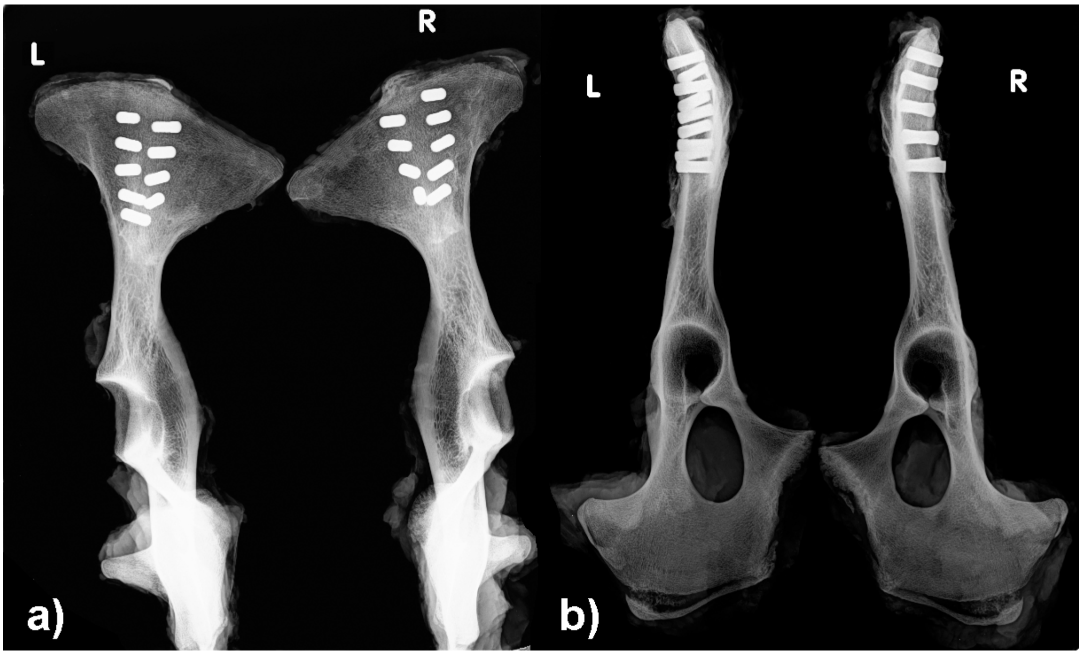

Fig.4: Those figures present radiological images of the pelvis separated in two halves after sacrifice. The left image shows a dorsolaterally projection, whereas for the right one a laterolateral projection was used. The implants can be seen as white and bright cylinders along the iliac crest on the upper part of the pictures.

Histological analysis

The histological analysis occurred according to well-established methods[28,42].

Briefly, assessment of bone-to-implant-contact (BIC) was made using the stained slides (Fig. 5-1).

For that, the length of the total implant surface (composed out of its two longitudinal surface segments, divided in a cortical and a cancellous portion) was measured, followed by measurement of the actual contact line between bone and implant separately on each side (presented as total BIC or divided in its cortical and cancellous portions). The BIC percentage was then calculated as the portion of implant surface with a direct bone-to-implant connection divided through the total implant surface length.

<u>Fluorescence sections:</u>

Fluorescence dyes bind to free calcium and by that enable the assessment of newly formed bone tissue over time. Visualization of the fluorochrome integration was made possible with the aid of a fluorescence microscope (Leica microscope SM 6000 B and DFC 350 FX R2 digital camera. Leica Microsystems AG, Heerburgg, Switzerland) and special filters (L5 for calcein green and N3 for xylonol orange). Fluorescence areas were evaluated descriptively for dye integration for each implant type in accordance to time (Fig. 5-2).

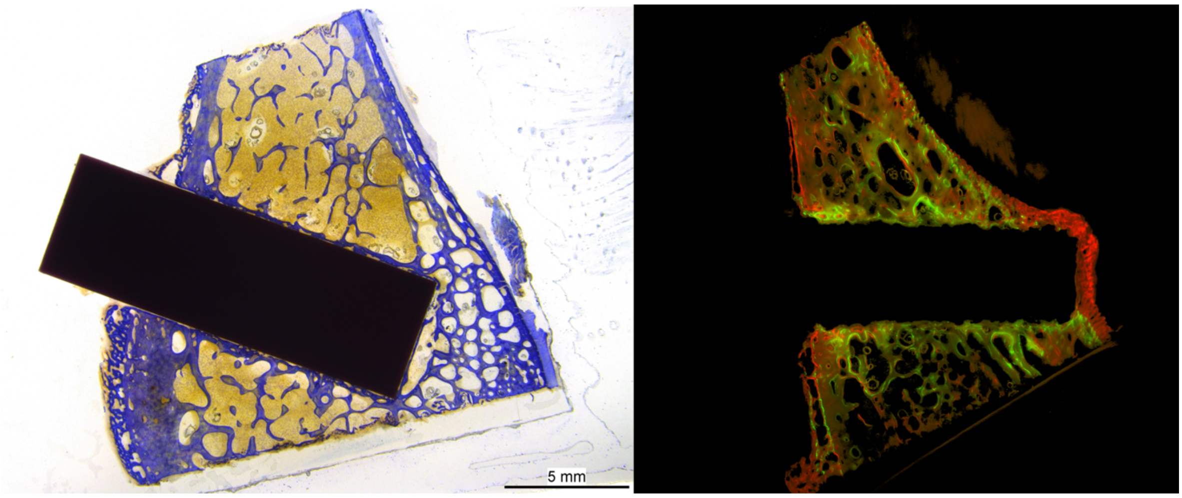

Fig.5: A macroscopical overview picture demonstrating histological images of a single bone-implant complex. The left figure presents the ground sections used for BIC evaluation. These were stained with toluidine blue. The left edge of the bone is the lateral cortex, the right one illustrates the medial cortex. The black bulk represents the implant (cut longitudinal in the midline), blue structures represent bone tissue and the yellow ones bone marrow. A microscopic picture used for the fluorescence evaluation of bone growth over time. The implant appears as an elongated black bulk in the middle of the picture. The green fluorescence color presents the calcein green dyes. The red-orange structures illustrate the dyes of xylenol orange. The right red colored edge of the bone tissue shows the lateral cortex whereas the left edge pictures the medial one.

Biomechanical tests

Push out tests were conducted similar to methods previously described[43]. Briefly, Implant-bone blocks were positioned in an Instron machine (Instron E10000 electrodynamic testing machine, Instron Corp. High Wycombe, UK) and visually centered under the pushing pin to ensure a uniform radial distance between the implant and lower support, consistent with recommendations of Dhert et al[44] (figure 6). The actuator of the Instron was advanced slowly until a preload of 0.2 N was achieved. The actuator was then advanced at a constant displacement rate of 1 mm/min and the load and displacement data were recorded continuously to a PC at 20 Hz. Push out force (N) was determined from the maximum force applied until failure of the bone / implant interface, or in the case of no clear maximum, the

0.2% offset yield value was calculated. Maximum structural stiffness (N/mm) was calculated from the slope of the linear section of the load – displacement curve.

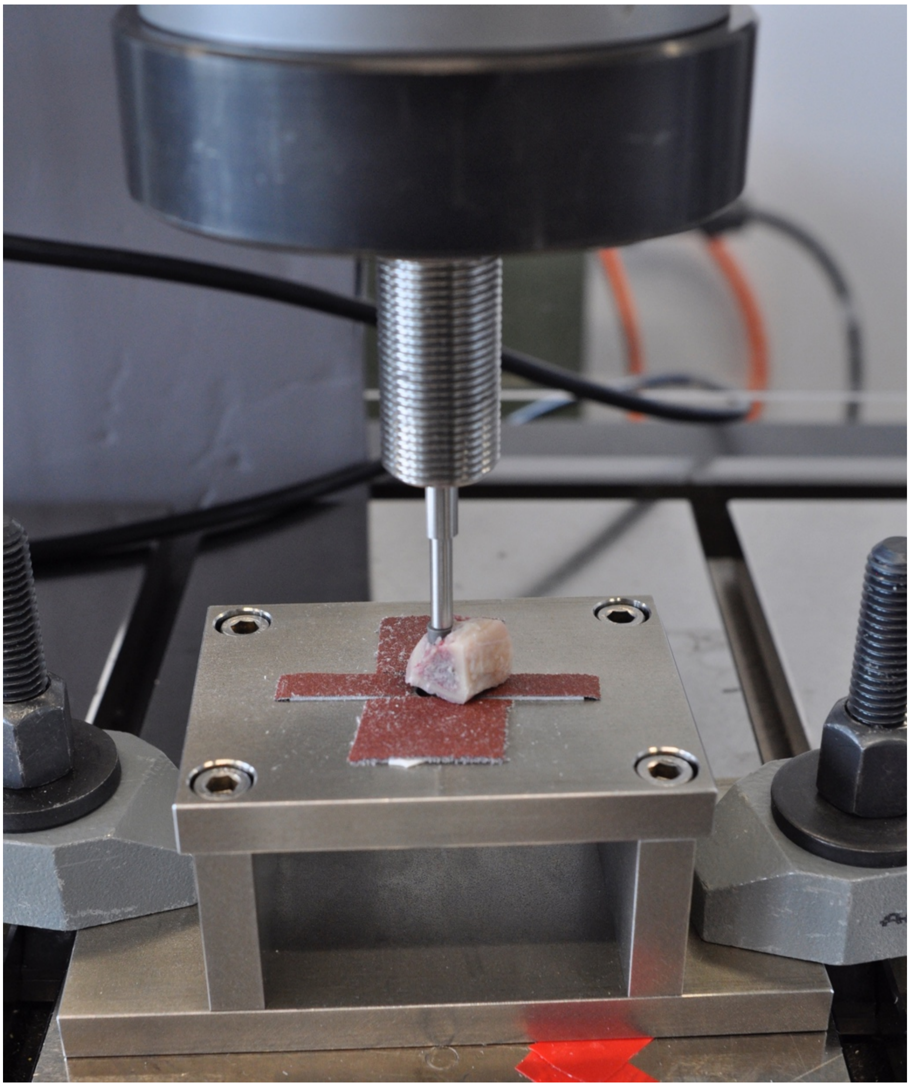

Fig.6: This picture shows the push out testing. A bone-implant block was placed in the middle of the working surface with its implant head showing towards the floor through a hole. The pushing pin of the Instron machine was then positioned directly on the distal end of the implant to allow pushing out the implant through the hole in a constant rate.

Statistical analysis

Statistical evaluation of biomechanical tests and BIC measurements was performed using the SPSS software (SPSS Statistics, Version 24.0). Mean values and standard deviations were calculated. A factorial analysis of variance (ANOVA) was chosen to test for statistically significant differences, with Bonferroni post-hoc tests for comparison of individual differences between groups. *P*-values<0.05 were considered statistically significant.

RESULTS

Surgery and postoperative period

All surgeries went well and the recovery period was uneventful. Furthermore, two days after surgery up until sacrifice all animals presented no unusual clinical signs such as discomfort, inflammatory or reduced water and food intake.

Macroscopic and radiologic evaluation

Throughout slaughtering and sample retrieval, no signs of inflammation, hematoma, foreign body reaction or impaired healing were identified. Two animals showed mild excessive growth of bone tissue, partially covering some of the implants. Implants were found to be well fixated in to their surrounding bone tissue and still in place. Those macroscopic observations were further confirmed by the radiologic imaging. In addition, no signs of bone resorption or fractured bone were diagnosed. The findings shown by the microradiographs were in accordance with the other histological evaluation. As a result, the microradiographs were not further analyzed.

Histomorphometrical evaluation (Bone to implant contact= BIC)

The bone to implant contact (BIC) measurements of the longitudinal implant interfaces, divided in cortical and cancellous bone regions are summarized in table 2.

In general, all TI's yielded high BIC values compared with the RI's.

Supporting the results of the biomechanical tests, Coating C presented the highest total BIC values compared to the RI's with a high statistically significance ($p<0.001$). It also showed a tendency of higher total BIC means among all other TI's. The measurements of total BIC also revealed statistically significant differences between coating B and both RI's ($p=0.028$ (RI-1); $p=0.041$ (RI-2)).

Being the coating with the second highest total BIC mean value, group B was followed by coating D, coating A, RI-1 and RI-2, all in ascending order. Nevertheless, no further significant differences were observed in between the different groups.

The evaluation of the cortex as well as the cancellous portion of the BIC revealed similar order to that of the total BIC.

Implant type	BIC total [%]	BIC cortex [%]	BIC cancellous bone [%]
TI-A	27.21 ± 03.22	28.92 ± 09.55	26.59 ± 03.03
TI-B*	39.90 ± 18.82	51.66 ± 30.21	37.56 ± 18.48
TI-C**	49.90 ± 13.36	53.89 ± 28.52	50.97 ± 17.45
TI-D	35.38 ± 10.55	34.92 ± 31.14	34.36 ± 13.53
RI-1	13.68 ± 12.33	07.23 ± 13.95	14.59 ± 13.46
RI-2	12.77 ± 16.20	18.93 ± 30.73	10.71 ± 12.27

Tab. 2: This table describes the mean value and standard deviation of BIC Values (%) of total, cortical and cancellous bone. Coatings marked with one star (*) were statistically significant higher than RI-2. Coatings marked with two stars (**) showed higher statistically significance compared with both RI's.

Biomechanical testing (Push-out tests)

The results of the push-out tests are illustrated in Tab. 3 and Fig 7.

Two out of 36 implants were excluded from the statistical evaluation of the biomechanical testing.

The first of (coating D) was not surrounded by enough bone tissue due to inaccurate preparation during explantation. The second of (RI-2) was not positioned correctly in the testing machine, resulting in test failure.

All four TI groups yielded high values compared to both RI's with low values.

Overall differences between groups for push out testing were significant at $p=0.000$. When compared individually, statistically highly significant differences could be found between Coating C and both RI's ($p<0.008$).

In addition, Coating B revealed statistically significant higher push out results compared to RI-2 ($p<0.04$).

Group	N	Minimum Force Destructive [N]
TI-A	6	716.75 ± 333.20
TI-B*	6	945.97 ± 399.87
TI-C**	6	1038.86 ± 385.16
TI-D	5	629.76 ± 313.31
RI-1	6	354.13 ± 138.17
RI-2	5	106.06 ± 63.30
Total	34	647.45 ± 428.76

Tab. 3: This table shows the mean values and standard deviations during biomechanical testing. Coatings marked with one star (*) were statistically significant higher than RI-2. Coatings marked with two stars (**) showed higher statistically significance compared with both RI's.

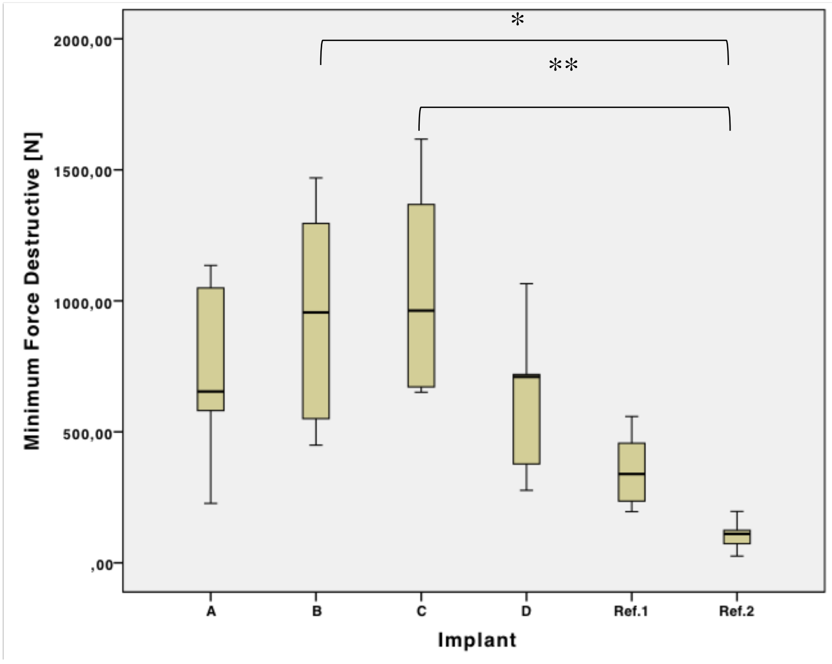

Fig. 7: This box-plot presents the values obtained by the push out tests. The dark black lines stand for the mean values. The region inside the boxes presents the main value distribution whereas the vertical lines above and under show the outliners. The X-axis describes the different implants whereas the Y-axis describes the destructive force in N. Coatings marked with a star (*) were statistically significant higher then RI-2. Coatings marked with two stars (**) showed higher statistically significance compared with both RI's.

Fluorescence

Administrated two weeks after surgery, calcein green showed a similar distribution in all four sheep without noticeable differences among the groups. The fluorescent marker was visible over the entire bone tissue of the specimen, though mainly concentrated in the areas directly around the implants (near the implant`s surface) respectively in the cancellous bone.

In contrast, xylenol orange, the second administered dye marker three dyes prior to sacrifice, presented a slightly different distribution. It exhibited the highest concentrations in the cortical regions.

DISCUSSION

It was the goal of this study to compare the osseointegrative properties of four novel implant coatings with two different uncoated RI's in a sheep pelvic model.

Results of both biomechanical and histological analysis have proven all coated TI's to achieve solid osseointegration. Moreover, titanium implants with an additional Ti-HA coating (Coating C) revealed a statistically significant (BIC $p<0.001$, Push out test $p<0.008$) higher osseointegrative potential in comparison to both uncoated RI's.

The literature describes several different animal models[45,46] for exploring the osseointegrative abilities of implants. Most commonly, however, especially rabbits, dogs or mini pigs are used[47]. They have the advantage of being easy to handle and their anatomy, biology and physiology are well known to the world of science due to their long time use in various fields of research. Nevertheless, they also come with some limitations, such as ethical issues or anatomical constrains as of the number and sizes of devices suitable for implantation. Whereas for example rabbits and mini pigs exhibit a good economic advantage over many other animal models[47-49], dog models present similar bone morphology to that of humans, though possessing a bone-remodeling rate of up to 3 times faster than that of humans[47,50]. Another alternative for testing implant materials is the pelvic bone model of sheep. It exhibits numerous benefits, as demonstrated in previous studies. It allows the implantation of up to 18 implants per animal, consequentially enabling the reduction of the number of animals used for the experiment (economical, animal welfare and ethical aspects). This also allows the implantation of up to 10mm long implants and enables a good separate evaluation of cortical and cancellous bone. Another positive aspect of this model is the similar bone metabolism rate to that of humans making it a good model when dealing with translational medicine research[2,51,52]. Those advantages were successfully validated by this current study.

Concerning the biological and biomechanical analysis, the results of this study coincide with the findings of Stubinger et al.[28]. In their study, the authors inserted PEEK and CFR-PEEK implants coated with a layer of Ti respectively a coating of HA on top of a Ti-layer in to the pelvic bone of sheep. In order to test their osseointegrative potential after 2 and 12 weeks they used histological (BIC and fluorescence) and biomechanical (push-out method) evaluation.

The authors were able to show that Ti- resp. Ti-HA coated implants demonstrate an increased osseointegration in comparison to uncoated RI's after 12 weeks. (Biomechanical testing: $p<0.001$; BIC: $p<0.02$, with the exception of one Implant-type). Furthermore, in this study the Ti-HA coating also reached the highest histological and biomechanical scores of osseointegration among all implants (push-out: 1250±280N, BIC: total BIC 69±23%, cancellous 67±24%). Yet even though overall results are similar, an additional influence of the bulk material on the final outcome cannot be ruled out completely. Generally, a possible explanation for the favorable outcome of Ti-HA coating over the other implant modifications is its surface topography. Bearing an outer layer of HA, it has a bone-like structure, which makes it bioactive and osseoconductive. This enables the implants surface to interact with the bone tissue more intensely and thus increases the bone-ingrowth[31,32,34,53,54]. Geesink et al[32]. showed already 30 years ago that HA has an excellent potential for promoting osseointegration. The authors have compared uncoated and HA-coated Ti implants in a femur model of dogs. Push-out tests revealed significant differences between uncoated implants (max. interface shear strength of 0.6MPa) and coated ones (63.9±1.7MPa) after six months. Histological analysis also showed increased bone growth, bonding and remodeling along the apatite layer. In the current study, coating C probably offered more contact area for bone tissue to grow in and on to[20,29,55]. That is possible due to the second layer of HA, filling out and minimizing the wider pores of the Ti surface. By turning them into smaller ones, it might offer the body cells a better environment for faster and more intense bone regeneration.

The other TI's (A, B and D) showed as well favorable results over the uncoated reference ones in the evaluation of BIC, though without a statistical significance. This corresponds with the findings of previous studies demonstrating that implant coating could have a positive effect on its osseointegrative capabilities[20,28,29,56]. One of such conducted by Stubinger et al[29] have used phosphoserine-tethered polyepsilon-lysine (PSD) coating of either sandblasted and etched (SE) or porous additive manufactured (AM) Ti-alloys. BIC-analysis showed a beneficial effect of the implant coating after 2 and 8 weeks on the rapid formation of new bone, hence osseointegration (SE-PSD: 46.7 ± 4.5; 61.7 ± 4.9 %, AM-PSD: 19.7 ± 3.5; 48.3 ± 15.6 %).

Results of the biomechanical test support prior studies suggesting the improvement of implant fixation by its coating. Such was found by Plecko et al[2] in their study in a pelvic sheep model. Coating cobalt chrome implants with titanium and zirconium improved the results of the biomechanical tests, implying that the coating is the one responsible for their enhanced osseointegrative properties. A further study in a sheep tibia model conducted by Devine et al[57] investigated the influence of titanium coating of CF/PEEK screws in comparison to uncoated implants. The biomechanical analysis (torque test) had shown increased values, from 2.4±0.8Nm (uncoated) to 3.4±0.8Nm resp. 4.9±1.4 Nm (depending on the coating method). A deeper look in to the figures of the push out tests in this current study reveals high means of standard deviation. Nevertheless, this is found with all implants and could be explained by the fact that all implants were randomly spread between all possible positions along the iliac crest. Depending on the position, the bone has different cancellous resp. cortex thickness.

Beneath the non-uniform distribution of cancellous and cortical bone between sheep the chosen animal model respectively study design also bears some other issues, which have to be critically and carefully addressed. The eight-week duration and minimal number of animals enabled an excellent set up for the assessment of the biomaterials osseointegrative properties and of the primarily implant stability. At the same time, it also enabled to maximize the use of numerous implants under the consideration of animal welfare. As it is, this animal model served this study well. Still, this kind of animal model cannot address the important aspects of long-term stability and load bearing (as applies to most dental and orthopedic implants). This would require a further, different research model.

For a future complete and ideal comparison of the biomaterials, the author also suggests using a higher number of animals, a third type of control item made out of pure tantalum (this wasn't possible due to the limited number of implants the iliac bone can sustain), as well as similar surface porosity and roughness for all TI's. This could also help validating the accuracy of this current study.

CONCLUSIONS

Overall, the present study disclosed that a porous and rough coating layer promotes osseointegration, delivering favorable results over uncoated implants. Additionally, its outcome suggests that an implant coated with a layer of HA upon a Ti one might furthermore increase its osseointegrative potential.

Finally, for a comprehensive clinical evaluation an experimental long-term study with more animals as well as a load-bearing situation is mandatory. ¨

CONFLICT OF INTEREST

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